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Short communication

Evaluation of the presence of the *bap* gene in *Staphylococcus aureus* isolates recovered from human and animals species

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Abstract:

The implication of biofilm in chronic bacterial infection in many species has triggered an increasing interest in the characterization of genes involved in biofilm formation. The *bap* gene is a newly identified gene that encodes the biofilm-associated protein, BAP, which is involved in biofilm formation in *Staphylococcus aureus*. So far the *bap* gene has only been found in a small proportion of *S. aureus* strains from bovine mastitis in Spain. In order to study the presence of the *bap* gene in *S. aureus* isolates obtained from other species and various locations, a collection of 262 isolates was tested by PCR, using published primers and dot-blot. The results indicated that none isolates carried the *bap* gene suggesting that the prevalence of this gene among *S. aureus* isolates should be very low.

Keywords: *Staphylococcus aureus*; *Bap* gene; Biofilm; Epidemiology

1. Introduction

In *Staphylococcus aureus* (*S. aureus*) the implication of biofilm in chronic infections in all animal species have triggered an increasing interest in the characterization of genes

involved in this biofilm formation. For example, the biofilm formation is important for virulence in mastitis (Baselga et al., 1993). A new gene (6,831 nucleotides) involved in biofilm formation (*bap* coding for a biofilm-associated protein, Bap) was identified in a small proportion of *S. aureus* from bovine mastitis (Cucarella et al., 2001). The bap protein is a member of proteins playing a role in biofilm formation in many bacteria. They share common structural features as they have a high molecular weight and contain a core domain of tandem repeats. These proteins confer upon bacteria the capacity to form a biofilm and play a relevant role in bacterial infectious process. Some of these proteins are contained occasionally in mobile elements (Lasa and Penades, 2006). In *S. aureus*, the *bap* gene is carried by a putative composite transposon inserted in SaPIbov2, a mobile staphylococcal pathogenicity island. *Bap* orthologue genes have been found in other staphylococcal species including *Staphylococcus epidermidis*, *Staphylococcus chromogenes*, *Staphylococcus xylosus*, *Staphylococcus simulans* and *Staphylococcus hyicus*. However, sequence analyses of the flanking regions revealed that these orthologue *bap* genes of these staphylococcal species were not contained in the SaPIbov2 pathogenicity island (Tormo et al., 2005).

The aim of the present study was to investigate the presence of *bap* gene in various *S. aureus* isolates recovered from human and different animal species. To do this a published PCR method was used (Cucarella et al., 2001) and results were confirmed by dot blot analysis.

2. Materials and methods

2.1. *S. aureus* isolates used in the study

Two hundred and sixty two *S. aureus* isolates associated with different diseases were recovered from various locations in France and different animal species (cows, sheep, goats, pigs, rabbits, poultry, horses, human) (table 1). The cow's isolates were a gift from Dr J.L Martel (AFSSA Lyon). Some sheep isolates were from ML De Buyser (AFSSA Maisons-Alfort). The goat's isolates were partly from Dr P. Mercier (AFSSA Niort). The pig, poultry and

rabbits isolates were from M.H. Bäyon-Auboyer (Departemental Laboratory Côtes d'Armor). The horse's isolates were a gift from Dr C. Collobert (AFSSA Dozulé). The human isolates were mainly from Dr H. Carsenti-Dellamonica (Hospital of Archet, Nice, France). All the remaining isolates were from AFSSA Sophia-Antipolis.

2.2. Detection of the *bap* gene by PCR

DNA extraction was performed using the DNeasy® Tissue Kit (Qiagen, Courtaboeuf, France) according the manufacturer's instructions with slight modifications. Lysostaphin (Sigma, St Quentin, France) were added (1mg/ml) for enzymatic lysis at 37°C for 2 h.

PCR were performed twice, using a primer pair (sasp-6m : 5' CCCTATATCGAAGGTGTAGAATTGCAC 3' and sasp-7c : 5' GCTGTTGAAGTTAATACTGTACCTGC 3') as described by Cucarella (Cucarella et al., 2004) to detect the *bap* gene. Amplification was carried out on a Mastercycler™ (Eppendorf, Hamburg, Germany) with Platinium® *Taq* DNA Polymerase (Invitrogen, Cergy Pontoise, France) under the following conditions: an initial 2 minutes denaturation step at 94°C; followed by 40 cycles each of 30 seconds at 94°C, 30 seconds at 55°C, and 75 seconds at 72°C; and a final step at 72°C for 5 minutes. A 971-bp PCR fragment was expected.

The primer pair (staur4 : 5' ACGGAGTTACAAAGGACGAC 3' and staur6 : 5' AGCTCAGCCTTAACGAGTAC 3') was used to target the 23S rDNA as described by Straub (Straub et al., 1999) to confirm the quality of each DNA extract and the absence of PCR inhibitor. The following conditions were used: an initial 5 minutes step at 94°C; followed by 30 cycles each consisting of 30 seconds at 94°C, 30 seconds at 58°C, and 75 seconds at 72°C; and a final step at 72°C for 5 minutes. A 1250-bp PCR fragment was expected.

A *bap* positive control strain V329 (Genbank accession no. AY220730, kindly provided by Dr J.R. Penadés, Spain) was used with each PCR run. Amplification products were electrophoresed in a 1% agarose gel containing ethidium bromide and visualized by transilluminantion under UV light.

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116 2.3. Dot blotting and hybridisation for the *bap* gene

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118 For the dot blotting, 141 isolates were randomly chosen among the two hundred and
119 sixty two isolates.

120 The dot blotting technique was described by Planchon et al. (Planchon et al., 2006).
121 Briefly, 50 ng of denatured DNA were spotted onto N + nylon membrane and treated
122 according to the manufacturer's instructions (Amersham Biosciences, Buckinghamshire,
123 England). The PCR product amplified with the primers sasp-6m and sasp-7c (Cucarella et
124 al., 2004), specific for the *bap* gene (971 bp long) from the V329 *S. aureus* strain, was used
125 as the probe. This PCR product was purified with a QIAquick® PCR purification kit (Qiagen,
126 Courtaboeuf, France), labelled with the DIG-High Prime® system (Roche, Neuilly sur Seine,
127 France). The hybridisations were done in DIG Easy Hyb® solution and the hybridised probe
128 was detected by the Dig colour detection® kit (Roche, Neuilly sur Seine, France) following the
129 manufacturer's instructions. *S. aureus* V329 (Genbank accession no. AY220730) was used
130 as positive control and the strain Mu50 (Genbank accession no. BA000017) as negative
131 control.

132

133 **3. Results**

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135 DNA extracted from 262 *S. aureus* isolates was tested for the presence the *bap* gene
136 by PCR, using the primer pair sasp-6m and sasp-7c, as indicated in the materials and
137 methods section. Although the positive control strain (V329) showed a band at 971 bp, as
138 expected, none of the tested isolates showed positive results. All isolates were also tested
139 for the presence of the *S. aureus* 23S DNA by using PCR to check for DNA quality, presence
140 of inhibitors of the PCR reactions and specificity. All isolates were found positive, thus
141 eliminating false negative results. An example of some PCR results is shown in figure 1.

142 Since it cannot be excluded that mutations or deletions could have occurred in the
143 primer pair region of the bap gene, 141 isolates were randomly selected and tested by dot

blotting. As illustrated in figure 2, all isolates were also found negative by using this technique, except the *bap* positive control strain V329. Therefore it is likely that the *bap* gene is lacking in all isolates of *S. aureus* tested in this study.

4. Discussion

The gene for the biofilm associated protein (*bap* gene) was not detected in the twenty hundred and sixty two *S. aureus* isolates of this study. These results are in agreement with previous surveys on *S. aureus* of human, bovine, rabbit and pig origins (Arciola et al., 2001; Vasudevan et al., 2003; Vancraeynest et al., 2004; Nitzsche et al., 2007) where the *bap* gene was not found in the *S. aureus* isolates recovered in these animal species of these studies. Our study is the first one with a wide range of *S. aureus* recovered from different animal species to show that the *bap* gene had not spread yet among *S. aureus*.

So far, the *bap* gene has only been found in *S. aureus* obtained from bovine subclinical mastitis in Spain (Cucarella et al., 2001). This gene is also present in other *Staphylococcus* species, including *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. simulans* and *S. hyicus* (Tormo et al., 2005; Planchon et al., 2006). But, the *bap* gene is not widely distributed in *S. aureus* isolates despite its presence in the pathogenicity island SaPIbov2, a mobile genetic element. Analysis of the *bap* flanking sequences revealed that *bap* is carried by a transposon-like element. The transposon is inserted in the pathogenic island SaPIbov2 which is mobile without the presence of a helper phage (Penadés, 2006). The ability to produce biofilms, associated with the presence of the *bap* gene has been shown to give growth and persistence advantage to isolates from bovine chronic mastitis (Cucarella et al., 2004). But, *S. aureus* is fully capable of forming biofilm in the absence of *bap* gene as shown for some isolates (Vautor et al., 2006). These isolates were associated with the well-known operon *ica*ADBC (manuscript in preparation). As producing biofilm is an advantage for virulence, it was the purpose of this study to look for the *bap* gene in *S. aureus* pathogenic isolates. We propose two hypothesis, to be confirmed, explaining why the *bap* gene had not

spread amongst others *S. aureus* strains: i) the *bap* gene must have been acquired recently by *S. aureus* in SaPIbov2 and consequently the gene has not been horizontally transferred yet ii) horizontal gene transfer is not easy between different *S. aureus* lineages due to their host specificities. The *S. aureus* lineages are supposed to be different between strains recovered from different animal species. The Sau1 type restriction-modification system found in *S. aureus* is one of specifics mechanism that controls the ability of mobile genetic element to spread between strains (Waldron and Lindsay, 2006).

In conclusion, this study indicated that none isolates carried the *bap* gene suggesting that the prevalence of this gene among *S. aureus* isolates should be very low. Finally, no evidence of horizontal transfer of the *bap* gene between *S. aureus* recovered from different animal species was found.

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